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BENDAZAC DECREASES IN VITRO GLYCATION OF HUMAN LENS CRYSTALLINS

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Purpose. Bendazac has been used as an anti-cataractogenic drug. It has been reported that this acts by preventing protein denaturation. In this study the ability of bendazac to inhibit in vitro glycation of human lens crystallins was evaluated.

Methods. Possible effects of bendazac were detected by incubation of WS crystallins with the reducing sugars glucose and fructose. The efficiency of bendazac was evaluated by means of selected parameters including: browning, glycation (measured as furosine content) and specific NTP-fluorescence.

Results. The results showed clearly that bendazac (bendazac L-lysine and sodium) inhibits the early stages of protein glycation, as well as the formation of fluorescent advanced glycation products. Bendazac lysine (20 mM) proved to be more effective in inhibiting fluorescence development (67% inhibition) than the corresponding sodium salt (35% inhibition). No significant differences were found with respect to furosine levels; about 40% inhibition was produced with either bendazac lysine or sodium salt.

Conclusions. Bendazac clearly inhibits glycation of human lens crystallins, as can be efficiently monitored by following specific changes in lens protein fluorescence. These results may constitute a new and relevant therapeutic approach to monitoring cataract development.

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INVESTIGATION OF A PUTATIVE XYLITOL PATHWAY, METABOLISM OF INOSITOL AND GLUCURONATE IN THE MAMMALIAN LENS

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Purpose Investigation of a possible pathway for the production of the polyol xylitol in the mammalian lens which does not involve "Aldose reductase" (AR) but does include myo-inositol, a metabolite thought to be involved in protecting the lens from both osmotic stress and protein glycation. The role of AR in production of polyols in the diabetic lens remains unproved. Kinetic, stoichiometric and crystallographic data suggest that AR is not a true enzyme. Therefore an alternative pathway for xylitol production is required. Furthermore, loss of protective myo-inositol in the lens by activation of this pathway could be a factor in the aetiology of diabetic cataract.

Methods Spectrophotometric and chromatographic assays have been used to demonstrate the presence of enzymes required for the putative pathway in both rat and bovine lenses. Radiolabelled inositol and cultured whole lenses have been used to investigate the flux of metabolites through the pathway. Glucuronate reductase, a key enzyme in the pathway has been purified and its steady-state kinetics and optimal pH and temperature have been determined.

Results Many of the enzymes required for the proposed pathway have been demonstrated in both bovine and rat lens homogenates, including D-Glucuronate reductase, Xylulose reductase, myo-Inositol-Oxygen-oxidoreductase and L-Gulonate dehydrogenase. D-Glucuronate reductase has been shown to exhibit first-order Michaelis-Menten kinetics with respect to both NADPH and glucuronate and to be inhibited by Thiol reagents. Appearance of radiolabel in fractions corresponding to glucuronate and xylitol suggest flux of inositol through the pathway in bovine lenses.

Conclusions We will summarise our evidence for the lens xylitol pathway to date and some of the properties of individual enzymes.

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MECHANISMS INVOLVED IN UV-A DAMAGE TO THE EYE LENS

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Purpose: To investigate the mechanisms involved in the damage caused by UV-A radiation on the eye lens.

Methods: Bovine lenses obtained from animals 2-3 years old were placed in specially designed culture cells for pre-incubation of 24h. The lenses were oriented so that the anterior surface faced the incident UV-A radiation source and were maintained in the cells during irradiation. After irradiation, lens optical quality was monitored throughout the culture period and lens epithelium, cortex and nuclear samples were taken for biochemical analysis.

Results: There is almost no change in the focal length variability (sharpness of focus) of the control lenses throughout the 9 days of the culture period. Irradiated lenses at levels of 25J/cm^2 to 35J/cm^2 of UV-A, demonstrated an increase in focal length variability 72 to 96 hours after irradiation. 48 to 96 hours later focal length variability returned to control levels. Damage to several lens epithelial enzymes appears before the optical damage. Transglutaminase activity in lens epithelium was not affected by UV-A radiation. However, the cross-linking enzyme in the lens cortex and nucleus was activated as a result of the irradiation and returned to control levels a few days later, at which time the lens recovered from the irradiation damage.

Conclusions: It appears that transglutaminase is involved in the mechanism by which UV-A causes damage to the eye lens.

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REGIONALITY OF PROLIFERATION AND UV-B INDUCED PROSTAGLANDIN SECRETION IN CULTURED LENS EPITHELIAL CELLS

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PURPOSE Control and local restriction of mitotic activity in the lens epithelium are not understood. Arachidonic acid, prostaglandin E2, and oxidizing radicals derived from the prostaglandin biosynthetic pathway have all been linked with regulation of lens cell proliferation after UV proliferation in addition to inflammation and cataractogenesis. Involved pathways are poorly understood. This study compares cell proliferation in central versus preequatorial lens epithelial cells and their UV induced PGE2 synthesis at different times after UV-B irradiation. METHODS Cultures of bovine lens epithelium (central and peripheral (preequatorial) region) were grown on uncoated dishes. Seeding density of passage I and II cells was 35 x 10^3 cells per dish (diameter 3.5 cm). UV-B radiation was 35 and 70 J/m^2. Sampled cells were taken from cultures in the exponential growth phase. The UV-B lamp had a maximal emission between 290-310 nm. After radiation (days 2, 4, 6) cells were left in culture for another 1 to 3 days (sampling days 3, 5, 7, 10) before cell numbers were evaluated. Viability tests were performed by adding 0.3 ml of trypan blue (Seromed) to 0.7 ml of the trypsinized cell suspension. Supernatants were collected (each sample representing 150000 - 600000 cells), extracted 2 times with hexane / ether (1:1), evaporated to dryness under N_2, and redissolved in acetone. Prostaglandins (PG) were then esterified using 4-Bromomethyl-7-methoxycoumarin (BrMMC), the acetone evaporated under N_2, and 35 µl acetone added to each. 15 µl of each sample was applied to a TLC plate (Merck HPTLC silica gel 60 F254) and the samples were run using a solvent system of ethyl acetate / hexane / diethylether / benzene / dioxane 48:33:9:8:2. Bands were visualized and quantified using digital image analysis (ScanPacK™, Biometra, FRG) under UV light. Supernatants from central and peripheral lens epithelial cells were collected at various times following UV irradiation. All cells were second passage, and results were given in amount of prostaglandin / cell. RESULTS Passage I cell cultures showed a higher peripheral cell proliferation, passage II cell cultures a higher central cell proliferation. UV-B led to a reduction of proliferation. In comparison to controls PG E_2 was lower 24 h after UV irradiation in both central and peripheral cells. In contrast, the amount of PG E_2 was found to be greater in UV irradiated peripheral cells than in controls 3 and 4 days after UV-B exposure. CONCLUSIONS PGE_2, the synthesis of which is decreased by free radical scavengers, inhibits cell proliferation in chick retinal pigment epithelial cells. Central and peripheral cells showed an origin dominated intensity of cell proliferation and prostaglandin secretion under all conditions investigated. The results indicate a decrease in lens epithelial cell proliferation and a corresponding increase in PG E_2 production at least 4 days following UV irradiation. Our findings confirm a role for both PG E_2 and free radicals in cell proliferation following UV irradiation. Although data further suggest that regional cell proliferation and prostaglandin secretion after UV-B irradiation are mutally linked, this relationship between regionality in cell proliferation and local variation in prostaglandin secretion cannot yet be clearly elucidated.